#19 Renewed

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**Confirmation No. 3112**
Kazuhisa HATAKEYAMA	:	Docket No. 2000-0644A
Serial No. 09/576,715	:	Group Art Unit 1655
Filed May 23, 2000	:	Examiner B. Forman
METHOD FOR GENE ANALYSIS	:	

AMENDMENT

THE COMMISSIONER IS AUTHORIZED TO CHARGE ANY DEFICIENCY IN THE FEES FOR THIS PAPER TO DEPOSIT ACCOUNT NO. 23-0975

Assistant Commissioner for Patents,
Washington, D.C. 20231

Sir:

Responsive to the Official Action dated January 25, 2002, the time for filing thereto being extended for one month in accordance with the Petition for Extension submitted concurrently herewith, please amend the above-identified application as follows.

In the Claims:

Kindly cancel claims 11 and 12 without prejudice.

Please amend the claims as follows.

E1

1. (Thrice Amended) A method of gene analysis by detecting hybridization between a probe nucleic acid and a sample nucleic acid comprising a target sequence complementary to that of the probe nucleic acid, wherein at least one of the probe nucleic acid and the sample nucleic acid is DNA, said method comprising:

providing a substrate on which either the probe nucleic acid or the sample nucleic acid is immobilized,

adding the other non-immobilized probe nucleic acid or non-immobilized sample nucleic acid on the substrate, said other non-immobilized probe nucleic acid or non-immobilized sample nucleic acid being labeled with a fluorescent substance,

E1 performing hybridization of the probe nucleic acid and the sample nucleic acid in the presence of a sequence-non-specific double-stranded DNA-binding protein having a function to stabilize a complementary double-stranded DNA,

detecting the hybridization of the probe nucleic acid and the sample nucleic acid from the presence of said fluorescent substance, thereby producing a hybridization signal having a hybridization signal intensity, and

performing gene analysis based on the hybridization detected, said gene analysis comprising one or more steps selected from the group consisting of detecting deleted regions, detecting the presence or absence of a mutation, mapping gene location, detecting mismatch and complete match, and detecting nucleotide sequence of the sample nucleic acid.

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3. (Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein is derived from a hyperthermophilic bacterium.

E2 4. (Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein is derived from an archaebacterium.

5. (Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein is derived from a bacterium belonging to the genus *Sulfolobus*.

E3 cont

6. (Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein is derived from *Sulfolobus solfataricus*.

7. (Twice Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein is a Sso7d protein derived from *Sulfolobus solfataricus*.

E3

8. (Twice Amended) The method according to claim 1, wherein the sequence-non-specific double-stranded DNA-binding protein has a homology of 75% or more with the amino acid sequence of SEQ ID NO: 9.

E4

10. (Thrice Amended) The method according to claim 1, wherein the amount of the sample nucleic acid comprising the target sequence is analyzed based on the intensity of the hybridization signal obtained from the hybridization of the sample nucleic acid and the probe nucleic acid, said hybridization signal being identified by the presence of said fluorescent substance after hybridization.

13. (Thrice Amended) A test kit for detecting hybridization between a probe nucleic acid and a sample nucleic acid comprising a target sequence complementary to that of the probe nucleic acid according to the method of claim 1, which test kit comprises at least a sequence-non-specific double-stranded DNA-binding protein having a function to stabilize a complementary double-stranded DNA, and an immobilized probe nucleic acid or non-immobilized probe nucleic acid labeled with a fluorescent substance.

[Please add the following new claims.]

14. A method of gene analysis by detecting hybridization between a plurality of probe nucleic acids and a sample nucleic acid comprising a target sequence complementary to that of the plurality of probe nucleic acids, wherein at least one of the plurality of probe nucleic acids and the sample nucleic acid is DNA, said method comprising:

providing a substrate on which either the plurality of probe nucleic acids or the sample nucleic acid is immobilized,

adding the other non-immobilized plurality of probe nucleic acids or non-immobilized sample nucleic acid on the substrate, said other non-immobilized plurality of probe nucleic acids or non-immobilized sample nucleic acid being labeled with a fluorescent substance,

performing hybridization of the plurality of probe nucleic acids and the sample nucleic acid in the presence of a sequence-non-specific double-stranded DNA-binding protein having a function to stabilize a complementary double-stranded DNA,

detecting the hybridization of the plurality of probe nucleic acids and the sample nucleic acids from the presence of said fluorescent substance thereby producing a single or plurality of hybridization signals, each having a hybridization signal intensity, and

detecting a polymorphism in the target sequence by comparing the intensity of each hybridization signal.

Eq. cont.

15. A method of gene analysis by detecting hybridization between a plurality of probe nucleic acids and a sample nucleic acid comprising a target sequence complementary to that of the plurality of probe nucleic acids, wherein at least one of the plurality of probe nucleic acids and the sample nucleic acid is DNA, said method comprising:

providing a substrate on which either the plurality of probe nucleic acids or the sample nucleic acid is immobilized,

adding the other non-immobilized plurality of probe nucleic acids or non-immobilized sample nucleic acid on the substrate, said other non-immobilized plurality of probe nucleic acids or non-immobilized sample nucleic acid being labeled with a fluorescent substance,

performing hybridization of the plurality of probe nucleic acids and the sample nucleic acid in the presence of a sequence-non-specific double-stranded DNA-binding protein having a function to stabilize a complementary double-stranded DNA,

detecting the hybridization of the plurality of probe nucleic acids and the sample nucleic acid from the presence of said fluorescent substance thereby producing a single or plurality of hybridization signals, each having a hybridization signal intensity, and

Cont. detecting the nucleotide sequence of the sample nucleic acid by comparing the intensity of each hybridization signal.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Initially, Applicant wishes to correct the Examiner's understanding of Applicant's filing of January 7, 2002. The Examiner notes in item 1 of the January 25, 2002 Official Action that a Continued Prosecution Application (CPA) was filed on January 7, 2002. However, Applicant wishes to clarify to the Examiner that a Request for Continued Examination was filed on January 7, 2002 and not a CPA. Applicant respectfully requests the Examiner to note this correction in the next Official Action.

Claims 11 and 12 have been cancelled without prejudice and rewritten in independent form as new claims 14 and 15, and claims 1, 3-8, 10 and 13 have been amended. The claims have been amended and rewritten to more particularly define the present invention. Support for the claim amendments and new claims is readily apparent from the teachings of the specification and the original claims. Specific support for the claim amendments can be found on pages 6 (lines 16-22), 18 (lines 25-26), 19 (lines 15-17 and 24-25), and 25 (lines 7-21), of the specification.

Applicant wishes to note that other than the phrases "*sequence-non-specific*" and "*an immobilized probe nucleic acid or non-immobilized probe nucleic acid labeled with a fluorescent substance*", the changes to the claims are merely editorial in nature and should not be construed

to narrow the scope of the claims. Applicant also wishes to emphasize that claims 11 and 12 rewritten in independent form as new claims 14 and 15, have been effected to clarify certain embodiments of the present invention and should not be construed to narrow to scope of claim 1. In other words, since claims 11 and 12 originally depend on claim 1, it must be interpreted that the specific embodiments of new claims 14 and 15 are also encompassed within the scope of claim 1 even though claims 11 and 12 have been rewritten in independent form. Thus, the phrase "*a probe nucleic acid*" in claim 1 should be read to include one or more probe nucleic acids as customary under U.S. practice.

With regard to the rejection of claims 1-8 and 10-12 under 35 USC § 112, second paragraph, Applicant believes that each ground of rejection has been overcome by the amendments to the claims.

Specifically, claim 1 has been amended to recite the analytical steps noted by the Examiner. Further, claim 1 has also be amended to provide proper antecedent basis for the recitation of "*the intensity of the hybridization signal obtained from the hybridization*" in claim 10 by adding the phrase "*thereby producing a hybridization signal having a hybridization signal intensity*" as per the Examiner's sugggestion. In addition, claim 10 has been amended to more particularly recite the relationship between the hybridization signal and the fluorescent substance by replacing the term "*represented*" with the term "*identified*" which is consistent with the Examiner's suggestion. Lastly, new claims 14 and 15 corresponding to original claims 11 and 12, respectively, have been written to incorporate the phrase "*a polymorphism*" and to clarify how the plurality of probe nucleic acids is used as requested by the Examiner.

In view of the wording of the claim amendments and new claims, Applicant believes that this rejection can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1, 2 and 13, under 35 USC § 102(b) as being anticipated by Weininger et al. (USP 5,871,902, issued February 16, 1999), this rejection is deemed to be untenable and is thus respectfully traversed.

To constitute anticipation of the claimed invention, a single prior art reference must disclose each and every material element of the claim. Here, in this case, the cited reference fails to teach or suggestion the "***sequence-non-specific double-stranded DNA-binding protein***" of the present invention.

Weininger et al. disclose stabilizing binding between Target Nucleic Acid (TNA) and Probe Nucleic Acid (PNA) by using Target Binding Assembly (TBA) which can specifically bind to a specific sequence. In the reference of Weininger et al., TBA is a ***sequence-specific double-stranded DNA-binding protein having a function to regulated gene expression of transcription factor or repressor*** as described in Column 9, lines 44, of the reference. The kind of TBA is properly selected according to a specific sequence of Target Binding Region (TBR) included in TNA to be detected, and selected TBA is used in the method for detecting the presence of a target polynucleotide.

In contrast, as described on page 6, lines 16-22 of the specification, the double-stranded DNA-binding protein of the present invention is "*a protein which binds to chromosome of eucaryote or that of prokaryote strongly and concerns retention of higher-order structure of chromosome*", which means that it is a protein which binds to double-stranded DNA in a

sequence-non-specific manner. That is, in the method for gene analysis of the present invention, it is not necessary to select or prepare the double-stranded DNA-binding protein according to the target sequence to be detected or to optimize reaction condition. Therefore, the method of the present invention can be used to universally perform gene analysis on any target sequence.

Generally, there are 5 types in DNA-binding protein (see SEIKAGAKUJITEN (Biochemistry Dictionary), 2nd ed., page 853, 1990, Tokyokagakudojin, Tokyo, Japan, a copy of which (with English translation) is enclosed herewith):

(1) a double-stranded DNA-binding protein changing structure of DNA and regulating gene expression; the DNA-binding proteins belonging to type (1) include regulatory proteins concerning gene expression such as Cro protein encoded by λ phage, CI repressor, CRP of *Escherichia coli* (cAMP receptor protein or catabolite gene activator protein), lactose operon repressor and so on. Transcription factors such as AP1 and Sp1, which bind transcription enhancer of eucaryote, also belong to type (1).

(2) a single-stranded DNA-binding protein necessary for process of replication, recombination or repair;

(3) a protein binding to chromosome of eucaryote or that of prokaryote strongly and concerning retention of higher-order structure of chromosome; Representative proteins belonging to type (3) include, for example, histone protein. Histone forms nucleosome structure with chromosome DNA.

(4) DNA-directed ATPase such as DNA helicase;

(5) DNA topoisomerase changing conformation of DNA.

In the above-mentioned classification, type (1) is the sequence-specific double stranded DNA-binding protein, and type (3) is the sequence-non-specific double-stranded DNA-binding protein.

The double-stranded DNA-binding protein used in the present invention is type (3) while the double-stranded DNA-binding protein used in Weininger et al. is type (1).

As described above, the double-stranded DNA-binding protein of the present invention is clearly different and distinguishable from that which is used in Weininger et al. If the double-stranded DNA-binding protein of Weininger et al. is used in the method of the present invention, it is necessary to select a proper kind of double-stranded DNA-binding protein according to the target sequence.

Thus, since Weininger et al. fails to teach the sequence-non-specific double-stranded DNA-binding protein of the present invention, this rejection can not be sustained and should be withdrawn.

With regard to the rejection of claims 3-8 and 10-12 under 35 USC § 103(a) as being unpatentable over Weininger et al. (USP 5,871,902, issued February 16, 1999) in view of Guagliardi et al. (Journal of Molecular Biology, 1997, 267: 841-848) and SwissProt (Accession No. 059631, December 15, 1998 and Accession No. P39476; P81550, February 1, 1995), this rejection is deemed to be untenable and is thus respectfully traversed.

To establish a *prima facie* case of obviousness, the cited references in combination must teach or suggest the invention as a whole and include all the limitations of the claims. Here, in this case, none of the cited references, in the combination set forth by the Examiner, teaches or

suggests the limitation "the sequence-non-specific double-stranded DNA-binding protein" recited in the claims.

As noted above, the "***sequence-non-specific***" double-stranded DNA-binding protein of the present invention is clearly different and distinguishable from the "***sequence-specific***" double stranded DNA-binding protein used in Weininger et al. Thus, since the combination of Weininger et al., Guagliardi et al. and SwissProt fails to teach or suggest all the limitations of the claims, a *prima facie* case of obviousness cannot be established.

In addition, it should also be noted that the combination of Weininger et al., Guagliardi et al. and SwissProt fails to teach or suggest the superior results obtained by the present invention. As argued in Applicant's previous response of November 30, 2001, the object of the present invention is to provide a method for gene analysis quickly and efficiently with high precision and high sensitivity by performing hybridization in the presence of a sequence-non-specific double-stranded DNA-binding protein having a function to stabilize a complementary double-stranded DNA, even when using fluorescent substance as the labeling compound. As it is well known in the art, fluorescent substance as a labeling compound is generally regarded as having lower sensitivity and lower S/N ratio than radioactive compound. Also, the present invention can perform gene analysis in a high-throughput manner even when using long chain nucleic acids. This is because the method of the present invention can be performed under high temperature, for example, at 60°C and at high speed.

In contrast, the cited reference, Guagliardi et al., discloses a method of gene analysis by using the protein Sso7d and performing a band shift assay with electrophoresis using radioactive compound as the labeling compound. This method cannot perform gene analysis in a high-

throughput manner since the gene analysis cannot be performed at high speed using a plurality of samples, due to the low detection sensitivity of hybridization in this method and the long time required for assay.

Thus, Guagliardi et al. teach a method of gene analysis with low sensitivity even when using radioactive substance as the labeling compound and do not disclose a method of gene analysis having high precision, sensitivity and speed using fluorescent substance as the labeling compound.

Therefore, since the combination of Guagliardi et al., Weininger et al. and SwisProt fails to teach or suggest all the limitations of the claims (i.e. *sequence-non-specific double-stranded DNA-binding protein*) and the superior results (i.e. high speed with high precision and high sensitivity) of the present invention., this rejection under 35 USC § 103(a) cannot be sustained and should be withdrawn.

With regard to the rejection of claim 13 under 35 USC § 103(a) as being unpatentable over Guagliardi et al. (M. Mol. Bio. 1997, 267: 841-848) in view of Stratagene (catalog, 1988, page 39), this rejection is deemed to be untenable and is thus respectfully traversed.

The test kit of the present invention comprises at least a sequence-non-specific double-stranded DNA-binding protein ***and an immobilized probe nucleic acid or non-immobilized probe nucleic acid labeled with a fluorescent substance***. The test kit of the present invention can perform gene analysis quickly with high precision and high sensitivity because of the combination of the sequence-non-specific double-stranded DNA-binding protein and probe nucleic acid labeled with a fluorescent substance.

Since the combination of the cited references (Guagliardi et al. and Stratagene) fails to teach or suggest all the limitations of the claims (i.e. *an immobilized probe nucleic acid or non-immobilized probe nucleic acid labeled with a fluorescent substance*) and the superior results (i.e. high speed with high precision and high sensitivity) of the present invention., this rejection under 35 USC § 103(a) also cannot be sustained and should be withdrawn.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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~ Reference Document ~

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SEIKAGAKUJITEN (Biochemistry Dictionary), 2nd ed., Page 853, 1990, Tokyokagakudojin, Tokyo, Japan

(I) 1) a double-stranded DNA-binding protein changing structure of DNA and regulating gene expression; 2) a single-stranded DNA-binding protein (SSB) being necessary for process of replication, recombination or repair; 3) a protein binding to chromosome of eucaryote or that of prokaryote strongly and concerning retention of higher-order structure of chromosome; 4) DNA-directed ATPase such as DNA helicase; 5) DNA topoisomerase changing conformation of DNA.

The DNA-binding proteins belonging to type 1) include regulatory proteins concerning gene expression such as Cro protein encoded by λ phage, CI repressor, CRP of *Esherichia coli* (cAMP receptor protein or catabolite gene activator protein), lactose operon repressor and so on. Transcription factors such as AP1 and Sp1, which bind transcription enhancer of eucaryote, also belong to this type.

(II) Representative proteins belonging to type 3) includes, for example, histone protein. Histone forms nucleosome structure with chromosome DNA.

スウイルス*科のマレック病ウイルス*は自然宿主にリンパ腫を起こす．サルのヘルペスウイルスには，自然宿主と異なる種類のサルなどに対してリンパ腫および白血病をひき起こすものがある．ポックスウイルス科にはウサギなどの繊維腫ウイルスがあり，そのなかに自然宿主でない異種のウサギに粘液腫をつくる粘液腫ウイルスがある．霊長類のポックスウイルスには，サルに表在性腫瘍をつくる Yaba サルウイルスやヒトの伝染性軟属腫ウイルスがある．ゲノムが小さなポリオーマウイルス属においていくつかのウイルスの DNA の全一次構造や，アデノウイルスの T 抗原*遺伝子の一次構造が決定され，その発現機構や遺伝子産物の解析が進んでいる．

科	属	おもな種（自然宿主）
パポーバウイルス	ポリオーマウイルス	ポリオーマウイルス（マウス），SV40（アカゲザル），BKウイルス（ヒト），JCウイルス（ヒト）
	パピローマウイルス	ウサギ(shope)パピローマウイルス，ヒトパピローマウイルス，ウシパピローマウイルス
アデノウイルス	マストアデノウイルス	ヒトアデノウイルス
	アビアデノウイルス	トリアデノウイルス
ヘルペスウイルス	ガンマ-ヘルペスウイルス亜科（属名未定）	マレック病ウイルス（ニワトリ），ヘルペスウイルス・サイミリ(リスザル)，ヘルペスウイルス・アテレス(クモザル)，ヘルペスウイルス・シルビラグス(ワタノヲウサギ)
	未　定	Luckeウイルス(カエル)(?)
ポックスウイルス	レポリポックスウイルス	繊維腫ウイルス（リス），野兎繊維腫ウイルス，家兎繊維腫ウイルス
	未　定	Yabaサルウイルス，みずいぼウイルス(*molluscum contagiosum*)(ヒト)

DNA 緩和酵素 [DNA relaxing enzyme] ⇀ スウィベラーゼ

DNA グリコシラーゼ [DNA glycosylase] DNA 中に生じた異常塩基とデオキシリボースの間の *N*-グリコシド結合を加水分解する反応を触媒する酵素である．その結果 DNA 上に脱塩基部位が生成する．異常塩基としては，dUTP が誤って重合されたり，シトシンの脱アミノ化によって生じたウラシル，アルキル化剤により生じた 3-メチルアデニン，アデニンの脱アミノ化により生じたヒポキサンチン，およびプリン環の開裂の結果生じたホルムアミドピリミジンなどがある．これらの異常塩基のそれぞれに対して特異性をもつ DNA グリコシラーゼが細菌から動物にいたるまで広く分布している．いずれも分子量は 2～3 万と小さく，補酵素を必要としない．本酵素の作用のあとは，脱塩基部位を認識して AP-エンドヌクレアーゼ*が一本鎖切断を行い，異常塩基の除去修復*反応が進行する．

DNA 結合タンパク質 [DNA-binding protein] DNA に親和性をもち DNA に特異的あるいは非特異的に結合するタンパク質の総称で，その機能から約 5 種類に分類することができる．1) DNA 構造に変化を与えて遺伝子発現を調節する二本鎖 DNA 結合タンパク質，2) 複製，組換え，修復の過程に必須な一本鎖 DNA 結合タンパク質(single-stranded DNA-binding protein, SSB), 3) 真核細胞あるいは原核細胞の染色体に強く結合し，染色体の高次構造の保持に関与するタンパク質，4) ヘリカーゼをはじめとする DNA 依存性 ATP アーゼ，5) DNA コンホメーションに変化を与えるトポイソメラーゼ．1)に属する DNA 結合タンパク質にはλファージにコードされるクロ(Cro)タンパク質，cI リプレッサー，大腸菌の CRP(cAMP 受容タンパク質*あるいはカタボライト遺伝子活性化タンパク質)およびラクトースオペロンリプレッサーなどの遺伝子発現の調節タンパク質が含まれる．真核細胞の転写エンハンサー内に結合する AP1*，Sp1* などの転写因子もこれに属する．2)はファージ，大腸菌から高等生物に至る多くの生物種から単離されている一本鎖 DNA 結合タンパク質でいわゆる SSB とよばれるものである．なかでも T4 ファージの遺伝子 32 タンパク質*は最初に発見された SSB で詳しく研究されている．3) に属するタンパク質の代表的なものは真核細胞のヒストンタンパク質である．ヒストン*は染色体 DNA とともにヌクレオソーム*構造を形成する．真核細胞ではそのほかに染色体に結合する HMG(high mobility group)とよばれる非ヒストンタンパク質*も数多く見いだされている．一方，原核細胞でもヒストンと類似している H タンパク質，DNA 結合タンパク質Ⅱ，HU タンパク質，IHF(integration host factor) などが見いだされている．これらのタンパク質の関与により細菌の染色体もヌクレオソーム様の構造をとりうる可能性も考えられる．中でも HU と IHF は染色体複製起点 (*oriC*) あるいはある種のプラスミドの複製に関与することが示されており，これらのタンパク質の結合による DNA の高次構造の変化が，DNA 複製を制御する可能性を示唆する．4) に属する DNA 結合タンパク質はさらに，a) 二本鎖 DNA を不安定化し，巻き戻しを促進する大腸菌の Rep タンパク質などのヘリカーゼ，b) DNA ポリメラーゼの結合と連続移動性(processivity)を高める付属タンパク質，c) 複製(n′タンパク質，DnaB タンパク質)，組換え(RecA, RecBC タンパク質)において機能する DNA 依存性 ATP アーゼなどに分類できる．最後の 5)にはトポイソメラーゼ I，φX174 遺伝子 A タンパク質，λ Int タンパク質などの切断-再結合タンパク質，DNA ジャイレース(トポイソメラーゼⅡ)などが属する．(⇀ DNA 巻き戻し酵素)

DNA 合成遅滞変異 [DNA-delay mutation] ファージ DNA 合成の開始を遅滞させる表現型を示す(T4)ファージ遺伝子(遺伝子 39, 52, 60, 58～61)の突然変異のこと．この突然変異を有するファージの DNA 合成は，実際には正常な時期に開始されるが，その合成速度は著しく低く，開始後 10～15 分になって初めて顕著な DNA 合成の増加が観察される．ファージリゾチームなどの後期タンパク質の合成も遅れて開始される．DD 変異(DD mutation)ともよばれる．

DnaG タンパク質 [DnaG protein] ＝プライマーゼ

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